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# Variables Affecting Latency of Rats Running on a Maze

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# **Variables Affecting Latency of Rats Running on a Maze**

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Honors Thesis

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## **Introduction**

Behavioral tasks are often used in animal models to observe certain phenomena. These phenomena can provide insight into cognitive processes and serve as an impetus for further studies. Means et al. (2000) studied time of day discrimination in rats by training them to go to one T-maze arm in the morning and the other in the evening. The researchers found that 63% of the rats were able to achieve this time of day discrimination. Moreover, they found that factors such as a random feeding schedule and the particular researcher overseeing the experiment did not affect the rats' learning of the task. The researchers concluded that time of day discrimination is likely a contextual stimulus based on both event memory and a trained circadian rhythm (Means et al., 2000). The same research team followed up with a study examining rats' time of day discrimination in choice tasks vs. go/no-go tasks. The rats were able to perform significantly better on go/no-go tasks than choice tasks. This finding persisted even when a random feeding schedule was put into place and temporal cues were masked. The researchers concluded that time of day serves as a temporal cue for go/no-go tasks, but it is not a helpful cue for choice tasks (Means et al., 2000). Lukoyanov et al. (2002) examined the effect of time of day on place learning and found that rats were able to incorporate the temporal information into their spatial schematic (Lukoyanov et al., 2002). These findings indicate that the time of training reinforced by food rewards may affect performance of the task. Lukoyanov et al. also found that food-restricted rats made fewer errors than rats fed at ad libitum (Lukoyanov et al., 2002). This indicates that food restriction and hunger levels at the time of training may affect performance of the task.

Morgan and Fields (1938) investigated the effect of food rewards on the speed at which rats complete a behavioral task. When multiple sessions of training were conducted in a day, a positive speed gradient was observed, indicating that the rats performed the task faster in

successive trials. Morgan and Fields also observed that extensive training largely extinguished this speed gradient, although a slight increase in speed during successive trials remained. However, feeding the rats reinstated the positive speed gradient, indicating that behavioral tasks may be completed with greater speed when food rewards are presented as a motive (Morgan & Fields, 1938).

Based on the research to date, I hypothesize the following:

Hypothesis 1 – Increased hunger levels will be associated with faster performance of a behavioral task. This is because, if the rats are hungrier, the food rewards will be a stronger motive for them to complete the task than if less hungry.

Hypothesis 2 – Training later in the day will be associated with faster performance of a behavioral task. This is because rats are nocturnal animals and later training times will be more consistent with their circadian rhythm than earlier training times.

Hypothesis 3 – Performance of a behavioral task will be faster for later sequential sessions (session three faster than session two, session two faster than session one). This is because the rats will learn within the first session that they will receive food rewards and this will motivate them to complete the task faster during subsequent sessions.

## **Methods**

### Subjects:

Seven F344 male rats were used in this experiment. The rats were placed in single housing cages as approved by the University of Connecticut IACUC (Protocol A14-012). The rats ranged in age from five to thirteen months.

### Apparatus:

A linear runway was made of black plexiglass. There were food cups at each end into which Bioserv sugar pellets were automatically dispensed. There were sensors on the maze that tracked the rat's movement. When the rat passed by the sensors on one end, a sugar pellet was dispensed into the food cup on the other end. The runway was placed in a small, dark room within a larger room. The sensors were connected to computers and equipment in the larger room. A computer program called RatOS was used to record the latency data for each rat and to dispense sugar pellets when appropriate (see Figure 1).



*Figure 1.* The runway run by the animals. The behavioral task was to run back and forth on this linear runway twenty times per session for three sessions. There were automated food cups on each end of the runway where sugar pellets were dispensed as a reward.

### Procedure:

This study used male F344 rats that were food-restricted to 85% of the ad libitum weight. They were trained to run back and forth on a linear track with food rewards at each end of the track. They were first trained to reach 60 runs in ten minutes, then were trained for three sessions of twenty runs each in five minutes or under. This task is similar to the go/no-go task examined by Means et al. (2000) since the rat does not have to make a choice between goal arms (Means et al., 2000). After reaching these criteria, Microdrives were surgically implanted into the rats' hippocampus, though this study examined pre-surgery data.

### Analysis:

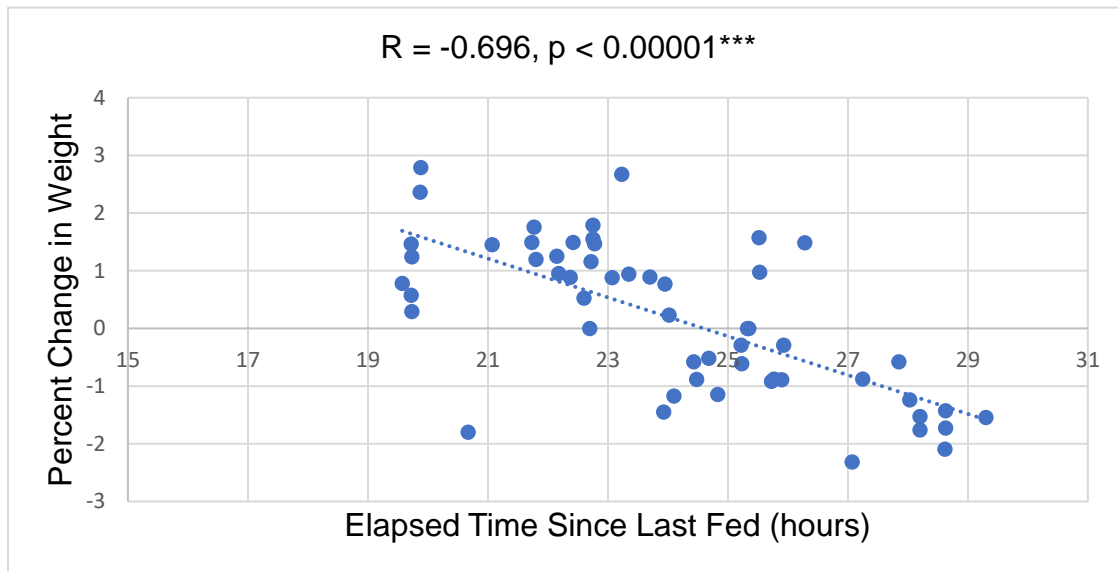
Data was collected using the recorded weights and performance times of each rat. The date and time of training, session latency, time of feeding, weight, and weight on the previous day was collected for each rat. The data collected included training days from December 2016 to May 2017. Inclusion criteria for the data included training days in which the rats completed three sessions of twenty runs each in under five minutes (300 seconds) before surgical implantation of the Microdrive. Exclusion criteria for the data included training days in which the rats did not complete three sessions of twenty runs each and training days that occurred after surgical implantation of the Microdrive. This resulted in a total of sixty-one days of viable data.

The time of feeding for each rat was calculated as the elapsed time in hours from the completion of session three the previous day to the completion of session three of the day in question. The percent change in weight from the previous day to the day in question was also calculated. A Pearson's correlation coefficient calculator was used to determine the correlation between the time of feeding and percent change in weight as a possible indicator of hunger level.

The correlations between time of feeding and latency (both average latency and individual session latency) and between percent change in weight and latency were also determined using a Pearson's correlation coefficient calculator. The significance of the session latencies for all three sessions was calculated using a repeated-measures ANOVA test. The difference between the session latencies for session one and session three was determined using both a one-tailed paired t-test and a two-tailed paired t-test.

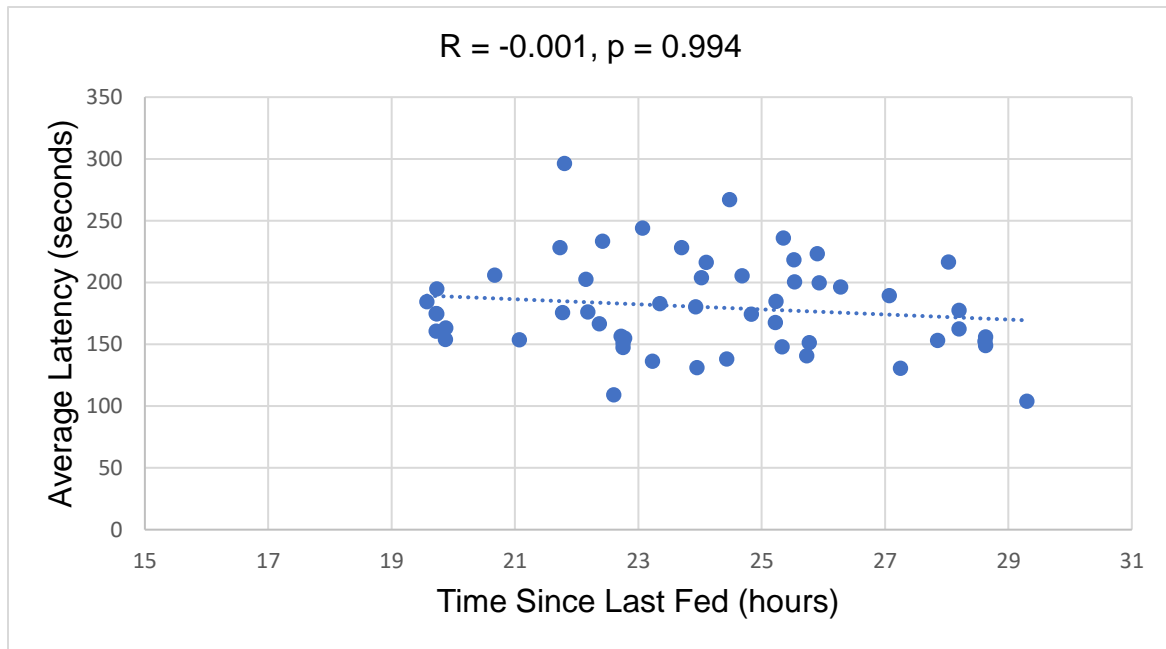
## Results

First, I examined the relationship between time of feeding and percent change in weight (see Figure 2). There was a significant negative correlation between percent change in weight and time of feeding for all rats ( $r = -0.696$ ,  $p < 0.00001$ ). This indicates that, the longer it has been since the rat was last fed, the more weight it loses. This is a measure of the rats' hunger levels.



*Figure 2.* The relationship between time elapsed between meals and percent change in weight. There was a significant negative correlation. The Pearson correlation coefficient was  $r = -0.696$  and the  $p$ -value was less than 0.00001, which is highly significant.

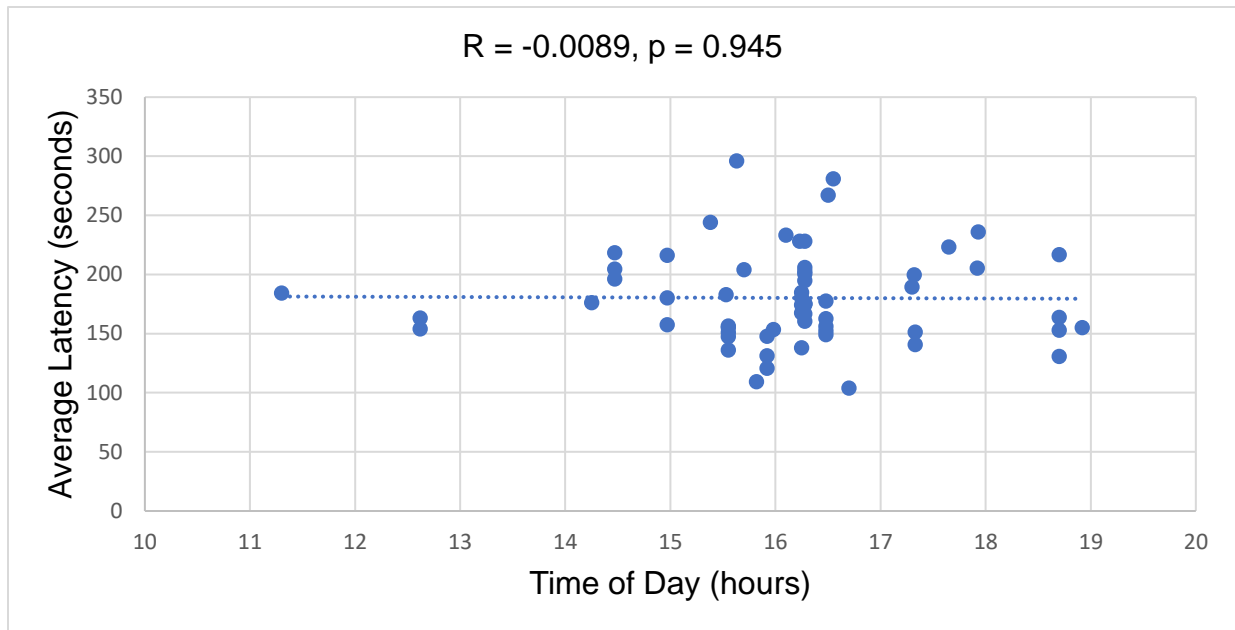
Next, I examined the effects of time of feeding and percent change in weight on latency (see Figure 3). There was no significant correlation between average latency (average across all three sessions) and time of feeding for all rats ( $r = -0.001$ ,  $p = 0.994$ ). There was also no significant correlation between average latency (average across all three sessions) and percent change in weight for all rats ( $r = 0.0549$ ,  $p = 0.693$ ). There was no significant correlation between individual session latencies (for all three sessions) and time of feeding. This indicates that there is no relationship between hunger levels and latency.



*Figure 3.* The relationship between time elapsed between meals and average latency (average across all three sessions). There was no significant correlation. The Pearson correlation coefficient was very close to zero at  $r = -0.001$  and the p-value was insignificant at  $p = 0.994$ .

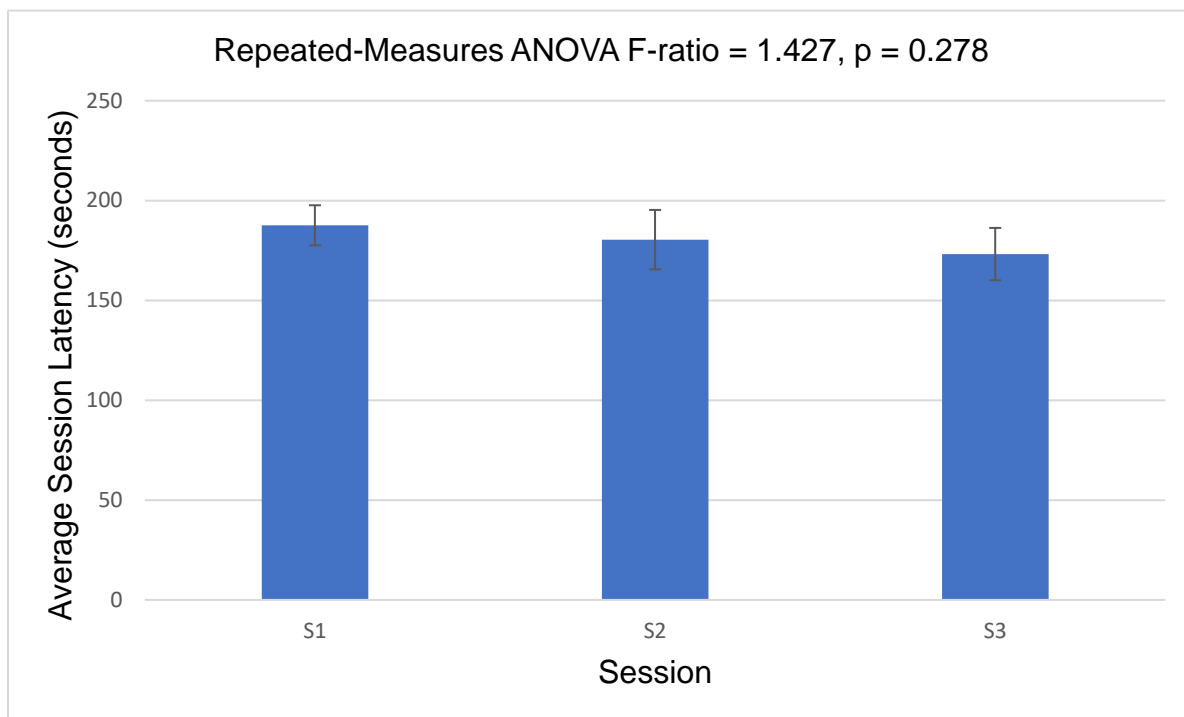
Next, I examined the time of day the animals were tested and its effect on latency. There was no significant correlation between time of training and individual session latencies (for all three sessions). There was no significant correlation between time of training and average latency (see Figure 4). This indicates that there is no relationship between time of day of training and latency.





*Figure 4.* The relationship between time of day and average latency (average across all three sessions). There was no significant correlation. The Pearson correlation coefficient was close to zero at  $r = -0.0089$  and the p-value was insignificant at 0.945.

Next, I examined for differences in latency across the three training sessions (see Figure 5). Since each rat had a different number of days of data, I calculated the average session latency for each session for each rat. The average session one latency of all rats was 187.6 seconds. The average session two latency of all rats was 180.4 seconds. The average session three latency of all rats was 173.2 seconds. The repeated-measures ANOVA test showed no significant difference among sessions ( $F\text{-ratio} = 1.427$ ,  $p = 0.278$ ). This indicates that session latency does not change significantly as the sessions progress.



*Figure 5.* The relationship between session number and average session latency. There no significant difference between session latencies. The repeated-measures ANOVA test F-ratio was 1.427 and the p-value was 0.278.

Next, I performed a two-tailed paired t-test on the average session latencies of session one and session three. The result of this test was not significant ( $t = -1.738$ ,  $p = 0.133$ ). This indicates that there is no difference between the latencies of session one and session three. Next, I performed a one-tailed paired t-test on the average session latencies of session one and session three. The result of this test showed a trend ( $t = -1.738$ ,  $p = 0.066$ ). This indicates that performance of session three is faster than performance of session one, but the data does not meet the criteria for statistical significance (p-value less than or equal to 0.05).

## Discussion

This study examined the effect of variables on task performance. Previous studies by Means et al. (2000) and Lukoyanov et al. (2002) have shown that temporal cues such as time of day have an effect on task performance by rats (Means et al., 2000; Lukoyanov et al., 2002). The

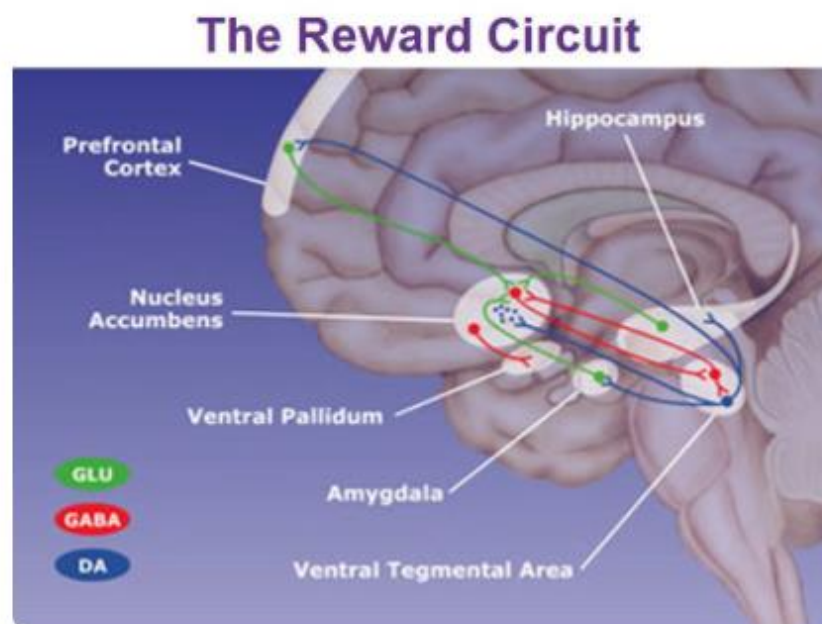
study by Lukoyanov et al. (2002) also showed that food-restricted rats performed better than rats fed at ad libitum, indicating that hunger levels may also affect task performance by rats (Lukoyanov et al, 2002).

Hunger levels can be estimated by time of feeding. Three methods can be used to approximate time of feeding: 1) the time elapsed since the rat's last meal (method used in this study), 2) the time two hours after the rat was weighed before training, and 3) the time of completion of session three. The results were the same regardless of the method used to define time of feeding.

Hypothesis 1 states that increased hunger levels will lead to faster performance on the runway (smaller latencies). There was a significant negative correlation between percent change in weight and elapsed time between meals. This finding indicates that percent change in weight is a good objective measure of hunger level; as more time elapses after a meal, the rats get hungrier and this can be observed as a greater decrease in weight. There was no significant correlation between percent change in weight and average latency or between time of feeding and average latency. This finding indicates that hunger levels do not have any significant effect on task performance, thereby disproving Hypothesis 1. Hypothesis 2 states that time of day of training will lead to faster performance on the runway (smaller latencies). There was no significant correlation between time of training and latency. This finding indicates that time of day does not have any significant effect on task performance, thereby disproving Hypothesis 2.

The results suggest that there is no change in motivation despite the rats' hunger levels or the time of day. The motivation pathway is the dopaminergic reward system, which consists of specific brain structures (most prominently the projections from the ventral tegmental area to the nucleus accumbens) that use the neurotransmitter dopamine to increase the occurrence of

pleasurable behaviors (see Figure 6) (Richardson et al., 2008). Richardson et al. (2008) examined nucleus accumbens dopamine transmission and found that it was sensitive to temporal cues (Richardson et al., 2008). Dopamine also encourages the consolidation of memories that are of motivational significance by input to the hippocampus (Arias-Carrion et al., 2010). The hippocampus is a brain structure located in the temporal lobe that is involved in processing episodic memory and spatial navigation (Nader, 2003). The hippocampus can be separated into the dorsal hippocampus and the ventral hippocampus. The dorsal hippocampus has been implicated in spatial navigation and the ventral hippocampus has been implicated in emotional memory (Moser & Moser, 1998). Therefore, temporal cues such as time of day should increase motivation to complete the task. However, this was not observed. This lack of change in motivation may be because, although food is used as a reward, it is not a significant motive.



*Figure 6.* The reward circuit consists of a variety of brain structures which are labeled in this figure. There is a dopaminergic synapse from the ventral tegmental area (VTA) to the nucleus accumbens. There is also dopaminergic input to the hippocampus from the VTA, which is responsible for the consolidation of motivationally-significant memories. There is also dopaminergic input to the prefrontal cortex from the VTA. The prefrontal cortex is implicated in decision-making. Therefore, more pleasurable behaviors are more likely to be repeated.

Lukoyanov et al.'s study found that food-restricted rats were able to discriminate time of day and this facilitated place-learning in a Morris water maze (Lukoyanov et al., 2002). The results from this study imply that the facilitation of learning observed in Lukoyanov et al.'s study is not applicable to the linear runway used in this experiment. Additionally, the Lukoyanov et al. study used a different strain of rats than this study, which can also affect the ability of the rats to perform the task.

Another temporal cue that should increase motivation according to Richardson et al. (2008) is session number. Hypothesis 3 states that subsequent session performances will be faster (smaller latencies) than previous session performances. The repeated-measures ANOVA test revealed no difference in latency for the three sessions. Therefore, the rats' motivation is not changing over the course of training on a particular day. This finding disproves Hypothesis 3. The two-tailed paired t-test showed no significant difference in session latency for session one and session three. The one-tailed paired t-test showed a trend for a decrease in session latency for session one and session three. This indicates that the rats' motivation may increase as they proceed through the sessions, thereby supporting Hypothesis 3. A larger sample size may provide the power necessary for the t-test to become statistically significant. Session two has the greatest amount of variance out of the three sessions. The standard error of the mean is 10.03 for session one, 14.89 for session two, and 13.12 for session three. Using a larger sample size may decrease this variance, resulting in significant results for the repeated-measures ANOVA test.

One of the strengths of this study is the use of objective measures such as time and latency. Even the subjective measure of hunger levels was objectified using time of feeding and percent change in weight. Another strength is sample size; sixty-one days of data was used. However, some rats had more days of data than others. This is a weakness of this study. The bias that rats

with more data could have over rats with less data was decreased by using the average session latency per session for each rat, but using an equal number of days of data for each rat may provide better results. This study could possibly be set up as a prospective study so that all necessary measures are meticulously recorded and equal amounts of data are collected for each rat. Additionally, the performance of food-restricted rats could be compared to that of rats fed at ad libitum.

In summary, hunger levels and time of training had no significant impact on task performance. There was a negative trend in session latency between session one and session three, indicating that subsequent sessions are performed faster than previous sessions. The findings from this study can be considered in future behavioral experiments involving food-restricted rats.

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